the kinetics of crystalline cellulose hydrolysis by cellulases has been investigated intensively so far, the mechanism of crystalline cellulose degradation still contains many mysteries. The main reason for the difficulty to understand the mechanism is the lack of analytical methods to monitor the enzymatic reaction at a solid/liquid interface. We here use high-speed atomic force microscopy (HS-AFM) to reveal how the enzyme molecules behave on the substrate. When glycoside hydrolase family 7 cellobiohydrolase from Trichoderma reesei (TrCel7A) was incubated with crystalline cellulose, many enzyme molecules moved unidirectionally on the cellulose surface with the velocity of 7.2 ± 3.9nm/sec but at some point the movement of individual molecules was halted, leading to appearance of traffic jams of enzyme molecules. The present results suggest that solving the traffic jams of productively bound cellulose is a key to enhance the hydrolytic activity of cellulases on crystalline cellulose.

2979-Pos Board B749

Towards Tracking Moving Single Molecules in Atomic Force Microscopy

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The atomic force microscope (AFM) is an invaluable tool for observing biological systems, due in part to its incomparable resolution as well as its ability to observe systems in their physiological environments and to measure mechanical properties directly. Its slow imaging rate, however, greatly reduces its applicability in recording fast-changing mechanisms. Such studies are of critical importance. For example, investigating the dynamics of protein motors and other macromolecules is essential for understanding and treating a variety of genetic diseases. Motivated by this, we are developing an approach to AFM centering on tracking rather than imaging. The scheme is primarily designed to follow the motion of a single macromolecule moving along a biopolymer. In approaching this problem, we consider that the presence of the moving macromolecule on its track results in a change in the apparent width of the track. Based on this, we have developed a high-speed width detector that rapidly determines the width by detecting the two edges of the sample during a fast scan. As a result, the motion of these single macromolecules is derived from direct tracking rather than a sequence of images. Such an approach promises a much higher temporal resolution than is achievable in time-lapse imaging.

2980-Pos Board B750

Encased Cantilevers and Alternative Scan Algorithms for Ultra-Gantle High Speed Atomic Force Microscopy

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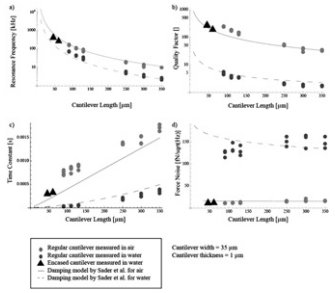
Live cells and many biological samples readily deform under the minimum force required to perform an AFM measurement precluding imaging at high temporal and spatial resolution. We reduced the force noise of the measurement by building a protective encasement around the cantilever. This keeps the cantilever is dry reducing the fluid viscosity and damping but allows the tip to probe the sample in solution. Encased cantilevers have exceptionally high resonance frequency, Q factor, and detection sensitivity and low force noise enabling gentle high speed imaging. Present raster scan techniques are poorly matched to the instrument limitations of Atomic Force Microscopy making data collection slow. We have used advanced image processing tools such as inpainting to recover high-resolution images from sparse quickly collected images to improve temporal resolution without applying more force or increasing bandwidth. We are also using spiral scanning to increase temporal resolution by allowing higher tip velocities without distortion. Inpainting or interpolation is used to quickly create images from the nongrided data.



2981-Pos Board B751

Ultrasensitive Force Spectroscopy with Tuning Fork Based Frequency Modulation

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The standard method that is used with atomic force microscopy to monitor mechanical properties of materials such as elasticity and adhesion is based on beam bounce technology. With such an approach there are two major problems: one is jump to contact and the other is adhesion ringing. Numerous methods have evolved for trying to resolve these problems from methods call pulsed force mode to peak force. However, what is desired is a smooth approach and retract from a surface or molecule where force measurements need to be implemented. Over the last few years, it has been realized that the best method of force feedback in atomic force microscopy is based on tuning fork force modulation but there have been few studies implementing these advances into the realm of force spectroscopy. In this paper, force spectroscopic analysis is implemented based on tuning forks and it is demonstrated that there is close to single pN force sensitivity. These efforts use the pioneering theory of Sader and Jarvis that has shown theoretically that is possible to derive accurate formulas for the force versus frequency in such Frequency Modulations methods [J.E. Sader and S. P. Jarvis, ''Accurate formulas for interaction force and energy in frequency modulation force spectroscopy'' Appl. Phys. Lett.84, 1801 (2004)]. It will be further shown that such normal force turning fork based force spectroscopy can readily be integrated with other chemical and structural tools such as Raman microSpectroscopy and Scanning Electron Microscopy.

2982-Pos Board B752

Measuring a Stabilization Constant Between Two Bio-Molecules using Atomic Force Microscopy

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Stabilization force constants are known to play an important role in biomolecular functions that carry out delicate structural conformation changes along a reaction coordinate during bio-molecular activation. Atomic force microscopy (AFM) has been used as a tool for probing protein-ligand interactions at the single molecular level. We developed a method that converts AFM force-distance curves into intermolecular force-distance curves. This method was applied to a model enzyme-inhibitor system of 5' methylthioadenosine/S-adenosylhomocysteine nucleo-sidase (MTN) and its transition state analogue homocysteinyl Immucillin A (HIA). Both the MTN and HIA molecules were attached to the sample and probe surfaces, respectively, through the flexible polymer polyethylene glycol. The stabilization force constant is found to be 0.235 N/m between MTN and HIA from the intermolecular force-distance curve, which is consistent with those measured by other techniques.

2983-Pos Board B753

Conducting Atomic Force Microscopy for Simultaneous Imaging of Structure and Ionic Current Through Nanopores

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Ionic currents through nanopores in both biological and synthetic materials play an important role in the function of the material. For biological systems, these pores are critical for normal physiological function and abnormalities lead to various disease states. The ability to measure the current through nanopores, while simultaneously relating their molecular and atomic structure, is currently limited in resolution. In order to perform structure-function measurements using an atomic force microscope (AFM), conducting cantilevered tips capable of measuring ionic currents in fluid were designed and fabricated using various techniques. Insulated tungsten wires with conducting tips were fixed to steel supports to create cantilevers for AFM imaging. Gold films in fluid were imaged with simultaneous electrical current measurements by the conducting cantilevers to reveal the topography of the film. These simultaneous recording of the current and the 3D structure demonstrate the conducting capabilities of the cantilever. Ionic currents through membrane filters were successfully measured through 20 nm pores in the membrane. The results from this technology show promise for future structure-function imaging of macromolecules, such as ion-channels in health and disease as well as for the synthetic nanopores for energy and environmental applications.